

Does Mating with Ginger Root Oil-Exposed Males Confer Fitness Benefits to Female Mediterranean Fruit Flies, *Ceratitis capitata* (Diptera: Tephritidae)?

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Abstract. Females of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), mate preferentially with males previously exposed to the aroma of ginger root oil, which contains the known male attractant α -copaene, over non-exposed males. As males of certain insect species are capable of absorbing and metabolizing air-borne molecules, this study examined whether females gain fitness benefits through mating with ginger root oil-exposed males, who might transfer oil-derived compounds during mating. Four components of fitness—female fecundity, female longevity, egg hatch, and egg-to-pupal development—were measured in the laboratory for females mated to exposed and non-exposed males. Despite female preference for oil-exposed males, mating with such males did not result in elevated performance for any of the four parameters measured. The evolution of female mate choice in *C. capitata* is discussed in light of these findings.

Key words: *Ceratitis capitata*, female choice, fecundity, longevity, fitness.

Introduction

Female insects may gain direct or indirect fitness benefits through mate selection (Kirkpatrick and Ryan 1991). Although the distinction between these categories is not always clear-cut, direct benefits usually derive from male-controlled resources or reduced search costs (i.e., energy expenditure and predation) and result in increased fecundity and/or survival of females and/or increased viability of the progeny. For example, males of certain Orthoptera provide nutrients to females at mating that are converted into eggs, resulting in higher lifetime fecundity (Vahed 1998). Alternatively, males of certain beetles (Eisner et al. 1996) and butterflies (Gonzalez et al. 1999) transfer chemicals at mating that are incorporated into the eggs and serve as defensive compounds against predators. Indirect benefits, on the other hand, derive from the male's genotype and result in increased viability and/or mating competitiveness of the progeny. Relative to direct benefits, indirect benefits are more difficult to detect, but an increasing number of studies have demonstrated increased offspring quality as a result of female mate choice in insects (Andersson 1994). For example, Hoikkala et al. (1998) showed that female *Drosophila montana* Patterson and Wheeler prefer males that produce a high-frequency song (generated via rapid wing vibration) and that the offspring of males producing the preferred song had higher survival than males producing non-preferred song types. In another instance, female crickets prefer to mate with males that have long calling bouts (Hedrick 1986). Because this trait has high heritability, females that select such males are likely to produce sons that also have long calling bouts (Hedrick 1988).

The Mediterranean fruit fly (or medfly), *Ceratitis capitata* (Wiedemann), displays a lek mating system (Prokopy and Hendrichs 1979, Arita and Kaneshiro 1989) in which males defend leaf territories (lacking resources critical to females), attract females via pheromone

signaling, and perform complex courtship as a prelude to copulation. Female mate choice appears to shape the mating system: females are free both in their selection of leks and territories for visitation and in their ultimate choice of mating partners. It is not known what factors determine female choice or what benefits female medflies receive through choice. Male signaling level has been shown to be positively correlated with both male mating success (Whittier et al. 1994, Whittier and Kaneshiro 1995, Shelly 2000) and male longevity (Papadopoulos et al. 2004). Thus, females may use signaling frequency as an indicator of male genetic quality and, by choosing males with high signaling level, may increase the probability of producing sons that likewise display increased signaling and longevity. Although this interpretation appears sound, Whittier and Kaneshiro (1995) failed to detect a positive association in a father-son regression of mating success, suggesting low heritability in male mating success (and, by inference, signaling intensity).

Alternatively, limited evidence suggests that female medflies may gain some direct benefit through mate choice. Whittier and Kaneshiro (1991) found that the fecundity of females that mated with virgin males was significantly greater than that observed for females mated to non-virgins. These authors suggest that males transfer an oviposition-stimulating substance to females during mating and that the amount of this substance transferred declines with successive matings. However, results from two other studies confound this interpretation. First, data from another tephritid species (Sivinski and Smittle 1987) show that only a tiny amount of substance (1/10,000 of male weight) is transferred from the male to the female during mating. Second, in laboratory choice tests, Shelly and Whittier (1994) found that females did not discriminate between virgin and non-virgin males, thus suggesting a small role, if any, for male mating status in female mate selection.

Several recent studies (Shelly 2001, Shelly et al. 2002, McInnis et al. 2002) have demonstrated that female medflies show a strong preference for males previously exposed to the aroma of ginger root oil over unexposed males. Ginger root oil contains α -copaene, a sesquiterpene hydrocarbon found in many plant species and a known attractant to male medflies (Flath et al. 1994, Nishida et al. 2000). The behavioral mechanism underlying female preference for ginger root oil-exposed males is unknown but may simply reflect increased signaling of exposed males (Shelly 2001).

The purpose of this study was to determine whether female medflies gain direct fitness benefits through mating with ginger root oil-exposed males. The rationale for this study derives from the observation that male insects may be capable of absorbing air-borne molecules and subsequently metabolizing them (e.g., Hughes 1974). Albeit indirectly, this study thus examined the notion that (1) male medflies absorb certain volatiles of ginger root oil and then transfer these molecules or their metabolites to females during mating (perhaps as a component of the accessory gland substances) and (2) this transfer affects positively the fecundity or survival of females and/or the viability of the progeny. Four components of fitness—female fecundity and longevity, egg hatch, and egg-to-pupal development—were monitored in the laboratory for females mated to exposed and non-exposed males. The evolution of mate choice by female medflies is discussed in light of these findings.

Materials and Methods

Study animals. Flies used in this study were from a laboratory colony started with 200–300 adults reared from Jerusalem cherry (*Solanum pseudocapsicum* L.) collected in Hawaii Volcanoes National Park. The colony was maintained in screen cages and provided with ample food (sugar:yeast hydrolysate in a 3:1 ratio by weight), water, and oviposition substrate (perforated plastic vials containing small sponges soaked in lemon juice). Eggs were placed on standard larval medium (Tanaka et al. 1969) in plastic containers placed over

vermiculite for pupation. Adults used in the experiments were separated by sex within 24 h of eclosion, well before reaching sexual maturity at 6–7 days of age, and kept in 5-liter plastic buckets (100–150 flies per bucket) with ample food and water. The flies were maintained at 23–27 °C and 65–85% RH and received both natural and artificial light with a photoperiod of 12:12 h (L:D). When used in the present study, the flies were eight generations removed from the field.

Female fecundity and longevity. Females (all 7 d old) were mated to males that either were or were not exposed to ginger root oil (for brevity, ginger root oil-exposed males, and females that mated with such males, are hereafter referred to as treated individuals; non-exposed males, and females that mated with such males, are referred to as control individuals). To expose males, we placed a paper disk to which 20 µl of ginger root oil had been applied (using microcapillary tubes) on the bottom of transparent, plastic cup (volume 400 ml), introduced 25 males (7–11 d old), and then covered the cup with nylon screening. Male exposure was started between 0800–0900 hrs and lasted 3 h. Males were removed from the cups with an aspirator and placed in the plastic buckets with food and water. Male exposure was performed in an isolated room to avoid inadvertent exposure of control males.

Two days after male exposure, we mated females to treated or control males. Sixty females and 60 males of a given type were placed in plastic, transparent cages (so-called Boller cages, 30 x 30 x 40 cm) between 0800–0830 hrs, and mating pairs were removed continuously (by gently coaxing individual pairs into vials) over the next 3 h. Pairs were then left undisturbed to complete mating in the vials. Only females that mated for at least 90 min were used to estimate fecundity and longevity, because the amount of sperm transferred increases with mating time up to, but not appreciably beyond, 90 min (Farias et al. 1972, Seo et al. 1990, but see Taylor and Yuval 1999).

After mating, the females were placed singly in transparent, plastic bottles (volume 4 liters) for the remainder of the experiment (mated males were discarded). The bottles containing control and treated females were kept in the same room under the conditions described above. To minimize potential effects of handling on female fecundity and longevity, only females that reached a minimum of 12 d of age (i.e., survived at least 5 d in the bottles) were included in the analysis. Females were supplied with food (the sugar-yeast hydrolysate mixture), water, and a perforated plastic vial for oviposition. Food and water were changed every 2–4 d, and the vial was replaced every 2 d. Deaths were recorded daily. Eggs were removed from the vials and placed on wet blotter paper using a small paintbrush and then counted under a dissecting microscope.

Hatching success and egg-to-pupal development. To estimate hatching success (proportion of eggs laid that hatched), I obtained and maintained control and treated females following the above protocol. Females were held for 1 week (eggs deposited during this interval were discarded), and during the following week I counted and placed eggs on wet blotter paper (following the above procedure) within petri dishes and placed the petri dishes in an incubator (27 °C). The numbers of hatched and unhatched eggs were scored three days later. Hatching success was estimated two weeks after mating to allow for the possibility that sperm longevity differed between control and treated males. Data on hatching success were collected only for females that laid 30 or more eggs during the week (i.e., over three egg collections performed on alternate days).

The proportion of eggs that led to pupation was monitored for another set of control and treated females (obtained and maintained following the above protocol). Eggs were collected at 2-d intervals (following the above protocol) and placed on larval medium held in small containers (eggs were collected during the week following mating, because, as shown below, there was no difference in egg hatch between control and treated females during the second week after mating). These containers were placed individually in plastic cups (400

ml volume) covered with fine screen mesh and containing a 1–2 cm deep layer of vermiculite as a pupation substrate. After two weeks, pupae were sifted from the vermiculite and counted.

Statistical analyses. Fecundity and longevity were compared between control and treated females using the non-parametric Mann-Whitney test, because neither raw nor log transformed data met the normality criterion for parametric statistics. Proportional data describing successful hatching and egg-to-pupal development for individual females were arcsine transformed, and control and treated females were compared using the t-test (assumptions of normality and homoscedasticity were met for both data sets). All analyses were performed using SigmaStat Statistical Software (Version 2.0).

Results

No difference in fecundity was detected between control and treated females (Fig. 1a). Median fecundity was 80 eggs for control females ($n = 84$) compared to 69 eggs for treated females ($n = 81$, $T = 6904.0$, $P = 0.57$). Likewise, there was no difference found in longevity between control and treated males (Fig 1b). Median longevity was 21.4 d for control females ($n = 84$) and 23.1 d for treated females ($n = 81$, $T = 6999.0$, $P = 0.37$).

Hatching rate was nearly identical between treated and control females. On average, 77% ($SD = 15$, $n = 17$) of the eggs collected from control females hatched compared to 76% ($SD = 21$, $n = 18$) for treated females ($t = 0.2$, $df = 33$, $P = 0.81$). Similarly, there was no difference between control and treated females in the proportion of eggs that developed successfully to the pupal stage. On average, 52.4% ($SD = 27.7$, $n = 28$) of eggs produced adults for control females compared to 57.0% ($SD = 25.0$, $n = 29$) for treated females ($t = 0.70$, $df = 55$, $P = 0.49$).

Discussion

Because males were assigned haphazardly to the treatment categories, genetic effects were presumably minimal, and the data reflected the magnitude of direct fitness benefits derived from mating with ginger root oil-exposed males. As the data show, however, none of the measured parameters—female fecundity and longevity, egg hatch, or egg-to-pupal development—differed significantly between control and treated females. Consequently, I conclude that female preference for exposed males is not based on direct fitness benefits associated with the transfer of particular chemicals during copulation. The adaptive nature of this preference thus remains unknown, but two (non-mutually exclusive) explanations appear plausible.

First, the preference may reflect direct benefits arising from reduced time and energy costs associated with mate searching (Dawkins and Guilford 1996). That is, females may mate more frequently with exposed males, because such males signal more (Shelly 2001) and hence are more easily located. Although based on a direct and realistic cause-and-effect relationship, this explanation is weakened by data showing that, while exposed males obtained 2-4 times more matings than non-exposed males in field-cage trials, exposed males signaled only 1.3 times as often as non-exposed males (Shelly 2001). Increased signaling is therefore not fully responsible for increased mating success of exposed males, and other factors (e.g., courtship behavior) are also important.

Alternatively, the preference for exposed males could reflect reduced costs of mate searching but arise from an inherent sensory bias in females and not from increased male signaling. Exposed males may produce an olfactory signal (i.e., pheromone or general body scent) that exploits a preexisting sensory bias that evolved in a different context, a phenomenon

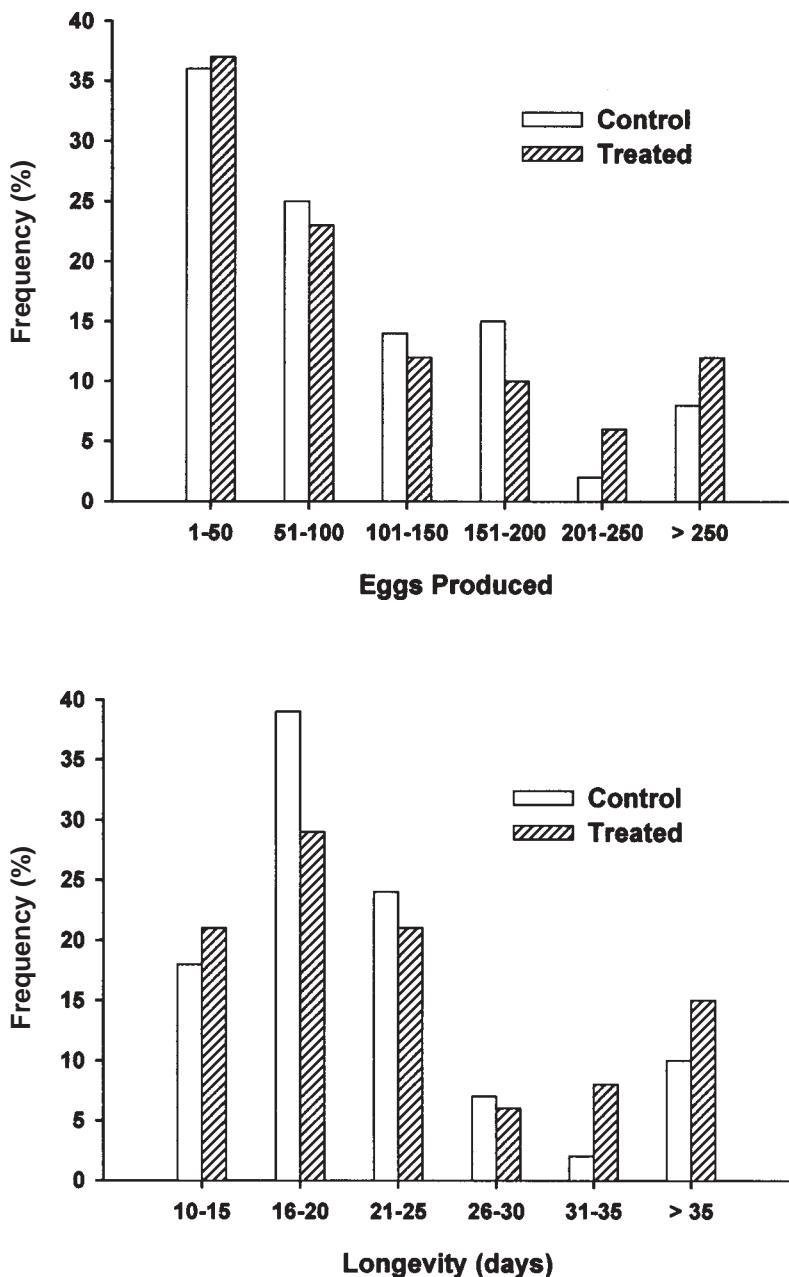


Figure 1. Egg production (top) and longevity (bottom) for *C. capitata* females mated to ginger root oil-exposed males (treated) or non-exposed males (control). See text for methods of data collection. Sample sizes: control = 84 females, treated = 81 females. Differences between treated and control females were not statistically significant for egg production or longevity.

termed a "sensory trap" by West-Eberhard (1984) or "sensory exploitation" by Ryan (1990). In this scenario, a lower sensory threshold to a specific odor evolved within females (perhaps in the context of searching for food or oviposition resources), and female mate choice reflects this heightened responsiveness to such odors.

Second, the emission of specific odors (as before, either the pheromone or general body scent) by exposed males may indicate a superior ability to locate natural sources of α -copaene (the presumed active agent of ginger root oil) in the environment. By selecting exposed males, then, females may increase the chances that their sons will have a high ability to locate sources of α -copaene and hence enjoy high mating success. This scenario depicts a case of runaway selection, whereby female choice provides indirect benefits via a trait that is arbitrary with offspring viability (Andersson 1994). This explanation still begs the question of why females prefer the particular scent broadcast by exposed males. The runaway selection scenario does not preclude the possibility that female preference for exposed males originated via sensory exploitation as described above. In fact, it is possible that this preference confers advantages through both increased mating competitiveness of her sons and reduced costs of mate searching.

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